

Guidance for Industry

Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

This draft document is intended to provide specific guidance for the development, evaluation, and application of mass spectrometric methods for confirming the identity of animal drug residues. It represents the current thinking of the FDA Center for Veterinary Medicine on the performance standards that qualitative mass spectrometry should meet for regulatory purposes. It elaborates the description of method specificity in CVM Guidance Document 3, *General Principles for Evaluating the Safety of Compounds Used in Food-producing Animals*, Part V, Guideline For Approval Of A Method Of Analysis For Residues, section B.1.

Comments and suggestions regarding this draft document should be submitted to the Dockets Management Branch (HFV-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852 after the publication of a notice of availability in the Federal Register. All comments should be identified with Docket Number provided in the Notice of Availability for this document.

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Draft Guidance for Industry: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues

This draft document is intended to provide specific guidance for the development, evaluation, and application of mass spectrometric methods for confirming the identity of animal drug residues. It represents the current thinking of the FDA Center for Veterinary Medicine on this matter. It does not create or confer any rights for or on any person and does not operate to bind the FDA or public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute and regulations.

INTRODUCTION

CVM develops, evaluates, and applies qualitative mass spectrometric methods for confirming the identity of animal drug residues. Methods developed in support of a New Animal Drug Application (NADA methods) are designed for residues of an approved new animal drug used in the approved manner. Methods may also be developed for unapproved new animal drugs or approved new animal drugs used in an unapproved manner (non-NADA methods). This draft guidance document describes the basic principles recommended by CVM for developing, evaluating and applying these methods.

The purpose of this document is to facilitate and expedite coordination between CVM and its stakeholders so these activities may be carried out in a consistent and timely manner. This draft document does not commit CVM to accepting a specific method or data package prior to reviewing the relevant data. This draft document is intended for technical professionals familiar with mass spectrometry. Please contact CVM for further information on this document or any technical explanations that may be necessary. For a historical perspective, please see the Bibliography. For definitions of terms used in this document, please see the Glossary.

This guidance document is applicable in the following areas:

1. Consultations on confirmatory methodology
2. Desk reviews of confirmatory procedures
3. Method trials or second-laboratory evaluations of confirmatory procedures
4. Development of confirmatory procedures
5. Desk reviews of data generated with confirmatory procedures

It is CVM's view that methods should fit the purpose. This document applies to work done for CVM's purposes, and does not necessarily apply to or invalidate work done for other purposes. This guidance applies only if a reference standard is available.

This guidance document should be used to help in the development of new methods, the review of methods submitted to CVM, and in the laboratory trial of methods submitted to

CVM. The document should also help in making decisions about appropriate methodology in various regulatory situations and ensuring consistency in work done for CVM's purposes. This document sets guidance standards and performance specifications as targets. CVM recommends that methods meet or exceed these standards.

GUIDANCE

Where CVM can predict that use of a new animal drug in food animals will likely result in the presence of drug residues in edible tissue of the treated animal, a full CVM Confirmatory Procedure should be developed and validated. For cases when a full procedure is unavailable and time does not permit a procedure to be fully validated, an *Ad Hoc* Confirmatory Package may be assembled. (See Glossary for these terms). The following sections list the specific elements that should be addressed in each case.

CVM Confirmatory Procedures are developed and validated in advance of their application. These methods should address each of the following points:

- I. Validation package from originating laboratory
 - A. Replicate samples
 1. Five Controls (may be subsamples from one source, but see part 1.F. below.)
 2. Five Fortified controls at tolerance/safe level
 3. Ten Residue-incurred, 5 at each of two levels
 - B. Demonstration of zero false positive rate.
 - C. Demonstration of <10% false negative rate at the tolerance or safe level is recommended (based on fortified and incurred samples). If this criterion cannot be met during method development, contact CVM.
 - D. Demonstration that suitable data can be acquired on more than one day. This helps to ensure data reproducibility.
 - E. Demonstration of non-interference by drugs approved in same species.
 - F. Demonstration of non-interference by matrix components in control samples from more than one source.
- II. **Method Description (Standard Operating Procedure, SOP)**
 - A. Scope of applicability
 - B. Method principles, including technique for mass spectral data acquisition.
 - C. Stepwise, unambiguous description of all reagents, apparatus, and steps.
 - D. Structure and full spectrum of marker residue.
 - E. Spectral data based on at least three structurally specific ions that completely define the parent molecule (may or may not include the parent ion), or more if non-specific ions are included. Use of water loss and isotopic ions are discouraged, but will be evaluated on a case-by-case basis.
 - F. Proposed fragment ion structures, consistent with fragmentation pattern.
 - G. Justification for specificity of selected ions or scan range.
 - H. System Suitability parameters.

- I. Confirmation criteria specified in advance (see Section III of this guidance).
- J. Operational criteria for repeat injection of same sample.
- K. Estimate of concentration limits for confirmation in matrix.
- L. Quality Control section (see Section IV of this guidance).

III. Confirmation criteria

These criteria are an expansion and updating of criteria that CVM has applied in the past. The new, expanded criteria are in response to the use of newer mass spectral techniques for regulatory confirmation. In CVM's judgement, all methods approved prior to issuing this document meet these criteria. Criteria should be specified in the SOP in advance.

- A. Comparison standard.
Comparison standard(s) should be analyzed contemporaneously. Preparation and analysis sequence of the comparison standard(s) should be fully described. Examples: first single injection prior to or after samples; average of all standards injected the day of analysis; average of two closest bracketing standards. If a matrix effect alters the spectrum or chromatography of a pure standard so that normal confirmation criteria cannot be met, a control extract-containing standard may be substituted for pure standard. Confirmatory methods that call for spiked control extracts for comparison should be justified.
- B. Chromatography/Mass Spectrometry
Any of the following chromatograms may be used: total ion chromatogram (TIC); reconstructed ion chromatogram (RIC); all single ion chromatograms (from scan, Selected Ion Monitoring (SIM) or Selected Reaction Monitoring (SRM)). Flow injection analysis is discouraged, but will be evaluated on a case-by-case basis.
 - 1. The chromatographic peak(s) should exceed a signal-to-noise (s/n) threshold of 3:1. A technique for estimating s/n should be described.
 - 2. A tolerance for retention time matching should be specified in the SOP. The tolerance should not exceed 2% for GC/MS or 5% for LC/MS, relative to the retention time of standard.
- C. Mass spectral matching.
Refer to Section II.E. for a discussion of structurally-specific ions. Confirmation criteria vary depending on the technique for mass spectral data acquisition.
 - 1. MS¹ full scan
The mass spectrum should include at least three structurally-specific ions. The spectrum obtained from a suspect compound should visually match the spectrum obtained from a contemporaneous standard. Since full scan data may include hundreds of significant data points for comparison, strict numerical criteria need not be

applied. [Matching within $\pm 20\%$ arithmetic difference on major ions is a useful rule of thumb, but is not required.] Library-search algorithms should not be used to confirm identity. The following elements apply when MS¹ full scan data are used:

- a. All structurally-specific ions identified in Section II.E. are present above a specified relative abundance.
- b. There is general correspondence between relative abundances or ranked abundances obtained for sample and standard.
- c. Ions other than from the target analyte can be explained (e.g. present in controls, blanks, etc.)
- d. If background subtraction is used, this should be specified in the SOP. The range used as background should always be indicated on the chromatogram.

2. MS¹ Selected Ion Monitoring (SIM).

- a. Relative abundances for three structurally-specific ions should match the comparison standard within $\pm 10\%$ (arithmetic difference, not relative difference). For example, at 50% relative abundance, the matching window would be 40-60%, not 45-55%.
- b. Relative abundances for four or more unique, structurally-specific ions should match the comparison standard within $\pm 15\%$.
- c. Relative abundances for more than three ions, which include ions due to isotopes or loss of water, should match the comparison standard within $\pm 10\%$.

3. MS¹ scan acquisition, SIM treatment.

If scan data is acquired, the data may be treated as for SIM acquisition (Section III.C.2.).

4. MS¹ partial scan

Criteria are the same as for full scan (Section III.C.1. above). All structurally-specific ions should appear in the scan range.

5. MSⁿ full scan

The spectrum obtained from a suspect compound should visually match the spectrum obtained from a contemporaneous standard. Since full scan data may include hundreds of significant data points for comparison, strict numerical criteria need not be applied.

- a. All structurally-specific ions identified in Section II.E. are present above a relative abundance specified in the SOP.
- b. There is general correspondence between relative abundances or ranked abundances obtained for sample and

- standard.
 - c. If a structurally-specific precursor ion completely dissociates to product ions after MS^n , the appearance of at least two additional structurally-specific product ions in the MS^{n+1} spectrum will be sufficient.
 - d. Ions other than from the target analyte can be explained (e.g. present in controls, blanks, etc.)
 - e. If background subtraction is used, the range used as background should be specified.
6. MS^n partial scan
Criteria are the same as for full scan (Section III.C.5. above). All structurally-specific ions should appear in the scan range.
 7. MS^n Selected Reaction Monitoring (SRM)
 - a. If a parent ion selected by MS^n is completely dissociated, and only two structurally-specific product ions are monitored in MS^{n+1} , the relative abundance ratio should match standard $\pm 10\%$.
 - b. If three or more structurally-specific ions are monitored, the relative abundance ratios should match standard $\pm 20\%$.
 8. MS^n scan acquisition, SRM treatment
If MS^n scan data is acquired, the data may be treated as for SRM acquisition (Section III.C.7.).

IV. Quality Control

- A. System suitability should be established before valid data can be obtained.
- B. At least one negative control and positive control should be run each day. The positive control should meet criteria and the negative control should fail criteria for the day's analyses to be valid.
- C. Sufficient blanks or negative controls should be analyzed after standards or positive samples to ensure that carryover does not cause a false positive.
- D. Operational criteria for repeat analysis of same sample: If a sample is analyzed but it can be shown that system suitability was not adequate during that analysis, the sample may be reanalyzed after taking steps to improve system performance and reestablish suitability.
- E. This document provides options for method developers, so that methods may be fit for their purpose. However, once a procedure is developed and the SOP prepared, a single set of confirmation criteria should be specified and used. Analysts should not substitute other criteria after analyses have

been carried out.

Ad Hoc Confirmatory Packages should meet or exceed the following minimal data recommendations:

Ad hoc data sets arise when new procedures are applied in response to unanticipated situations, when full confirmatory procedures are unavailable, and when time does not permit a procedure to be fully validated. CVM's confidence in *ad hoc* data packages is based on good quality assurance, good training, and high expertise in the laboratory. The following analyses are the minimum recommended for an *ad hoc* data package, and additional supporting data is strongly encouraged (see above). All recommendations for structural specificity (Section II.B-F.), confirmation criteria and recommendations for treatment of data (Section III.), and quality control (Section IV.) still apply.

- I. At least two controls should be analyzed. No control analysis should meet criteria (i.e., give a false positive). A true control derives from the same type of matrix, but is known to be free of the suspect compound. A surrogate control is from a similar matrix known to be free of the suspect compound, but which is used to simulate the same matrix. A survey control is from the same type of matrix, but is of unknown origin, and which has been analyzed repetitively and found to fail confirmation in every case.
- II. Control samples fortified with the suspect compound should meet confirmation criteria. At least two controls should be fortified at the suspect compound's tolerance or safe level. If the suspect compound has no tolerance or safe level, at least four fortified control samples should be prepared: two above and two near the suspect compound's estimated level.
- III. Sufficient replicate injections of standard should be made to establish system suitability.
- IV. A blank or negative control should be analyzed after a fortified sample or standard to demonstrate that carryover does not cause a false positive. Otherwise, blanks should be analyzed after each sample until the blank analysis appears free of standard.

BIBLIOGRAPHY

1. J.A. Sphon, *J. Assoc. Off. Anal. Chem.* 61 (1978) 1247-52.
2. T. Cairns, E.G. Siegmund, and J.J. Stamp, *Mass Spectrom Reviews* 8 (1989) 93-117.
3. T. Cairns, E.G. Siegmund, and J.J. Stamp, *Mass Spectrom Reviews* 8 (1989) 127-145.
4. J.B. Schilling, S.P. Cepa, S.D. Menacherry, L.T. Bavda, B.M. Heard, and B.L. Stockwell, *Anal. Chem.*, 68 (1996) 1905-1909.
5. W.G. de Ruig, R.W. Stephany, and G. Dijkstra, *J. Assoc. Off. Anal. Chem.* 72 (1989) 487.
6. Baldwin, R., et al, *J. Am. Soc. Mass Spectrom.* 8 (1997) 1180-1190 .
7. Bethem, R.A.; Boyd, R.K., *J. Am. Soc. Mass Spectrom.* 9(1998) 643-648.

GLOSSARY

<i>Ad Hoc</i> Confirmatory Package A	A data package accompanied by a conclusion that is supported by the data. <i>Ad hoc</i> data packages may be acceptable when a CVM Confirmatory Procedure is unavailable, and time does not permit a procedure to be fully validated. Examples of the need for such procedures include unanticipated misuse of an approved drug; unanticipated use of an unapproved drug, suspected presence of drug in unexpected tissue matrix, or sabotage of food products.
Confirmation	Unambiguous identification of a compound's presence by comparison to a reference standard (mass spectrometric).
CVM	FDA Center for Veterinary Medicine.
CVM Confirmatory Procedure	A procedure which CVM considers valid for regulatory analyses. This procedure should include a stepwise description of the method for evaluation by CVM. Such procedures can be developed in advance of their application because their need can be anticipated. Examples of the need for such procedures include the approval process for new animal drugs and preparation for surveys of suspected drug misuse. Such procedures are reviewed by CVM prior to a laboratory evaluation of the procedure at CVM. The evaluation consists of a sample set corresponding to Section I.A-D (see above).
Full Spectrum	Covering the mass range that encompasses all diagnostic detail, or, the full width of instrumental capability. For example, full spectrum may include both the molecular ion and low molecular

weight fragment ions.

Marker Residue	The residue selected for assay whose concentration is related to the concentration of the residue of concern in the last tissue to deplete to its permitted concentration.
MS ⁿ	Two or more stages of mass separation conducted sequentially.
Residue	Any compound present in tissues which results from use of a drug.
Safe Level	A level set by FDA for residues in edible tissues of treated animals, resulting from extralabel drug use, below which the agency does not have food safety concerns and that is based on available residue and metabolism information, or other appropriate scientific or regulatory criteria.
SOP	Standard Operating Procedure. A stepwise written procedure for carrying out an analytical method.
Structurally specific	Characterizing a compound-s molecular weight and/or unique substructure. For a molecule to be completely defined, the spectral data should be unique to that compound and none other.
Suitable data	Data acquired when system suitability has been met.
System Suitability	The fitness of analytical instruments for the purpose at hand, based on manufacturer specifications, instrumental Standard Operating Procedure, or specific requirements of the analytical method. Suitability may be established through verification of relevant instrumental parameters such as calibration, pressure, flows, temperature, multiplier gain, etc., or through verification of method-specific parameters such as signal-to-noise level for a known amount injected, peak shape, test spectra, etc.
Target Analyte	The chemical entity that a particular method is designed to detect.
Target Tissue	The tissue selected to monitor for residues.
Tolerance (Rm)	The concentration of the marker residue in the target tissue when the residue of concern is at the permitted concentration in the last tissue to deplete to its permitted concentration.